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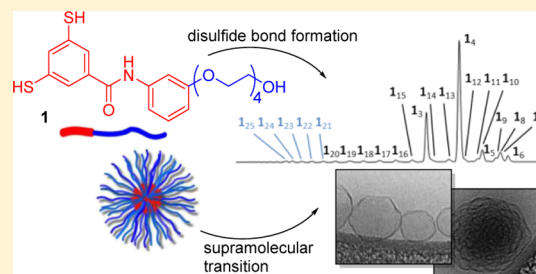
Emergence of Compartments Formed from Unconventional Surfactants in Dynamic Combinatorial Libraries

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Supporting Information

ABSTRACT: Assembly processes can drive the selection of self-assembling molecules in dynamic combinatorial libraries, yielding self-synthesizing materials. We now show how such selection in a dynamic combinatorial library made from an amphiphilic building block which, by itself, assembles into micelles, can yield membranous aggregates ranging from vesicles to sponge phases. These aggregates are made from a mixture of unconventional surfactant molecules, showing the power of dynamic combinatorial selection approaches for the discovery of new, not readily predictable, self-assembly motifs.



INTRODUCTION

Self-assembly is a powerful concept to access nano-sized structures that are organized with up to atomic resolution.^{1–3} Traditionally, access to such structures involved two separate steps in which the design and synthesis of the self-assembling molecule are followed by the assembly process in a separate second step. In many cases, the different conditions required for synthesis and assembly preclude their integration into a single process. However, with the advent of dynamic covalent chemistry,^{4–6} different chemistries are now available, which can be operated under conditions compatible with those required for assembly, allowing a systems chemistry^{3,7–10} approach to self-assembly where the synthesis of the assembling molecules and their self-assembly become intertwined. Specifically, assembly processes occurring in dynamic combinatorial libraries (DCLs)^{11–14} allow access to what we termed “self-synthesizing materials”.¹⁵ The idea is that upon formation of reversible covalent bonds between different building blocks, a diverse set of different potentially self-assembling molecules is formed, which continuously inter-convert by exchanging building blocks (Figure 1a). Library members that self-assemble are stabilized by the noncovalent interactions involved in the assembly process and the equilibrium shifts to amplify the assembling molecules at the expense of the other library members. This approach has allowed access to a variety of self-assembled structures, including fibers¹⁵ and sheets^{16,17} (for these morphologies, the process of self-assembly-driven self-synthesis is often autocatalytic, enabling access to self-replicators), micelles,^{18–22} and vesicles.²³ The dynamic combinatorial approach to self-synthesizing materials complements polymerization-induced self-assembly,^{24–26} in that, in the former, the assembling molecules are formed through reversible covalent bonds, whereas the latter typically involve kinetically controlled polymerization reactions. Reversibility ensures proofreading

that, in principle, enables selectively populating the most stable assembling systems. In the context of the origin of life and the de novo synthesis of life, the idea of obtaining self-synthesizing compartments (i.e., discrete supramolecular structures able to keep the molecules contained within them mobile) is of particular significance.^{27–32} Among different possible membranous structures,^{33,34} vesicles are particularly appealing, given their widespread occurrence in currently known life forms. Yet access to self-synthesizing vesicles has not progressed beyond proof of principle, targeting conventional double-chain surfactants.²³ We now report the formation of a series of different membranous architectures, including vesicles, by a mixture of nonconventional surfactants that are selected from a small DCL formed from a simple amphiphilic building block. These results demonstrate that the concept of self-synthesizing compartments extends well beyond canonical surfactant architectures.

RESULTS AND DISCUSSION

We reasoned that it should be possible to discover new vesicle forming surfactants in DCLs made from amphiphilic building blocks. Given that bilayer architectures are normally more efficient in shielding their hydrophobic interior from water than micelles, we expected that combinatorial selection experiments would preferentially lead to vesicles, even if the building block by itself would assemble into a different morphology. In order to test these hypotheses, we designed building block 1 which contains a hydrophobic aromatic core functionalized with two thiol groups, which can be oxidized to form a DCL of macrocyclic disulfides (Figure 1b). It also features a hydrophilic tetraethylene glycol chain that enables

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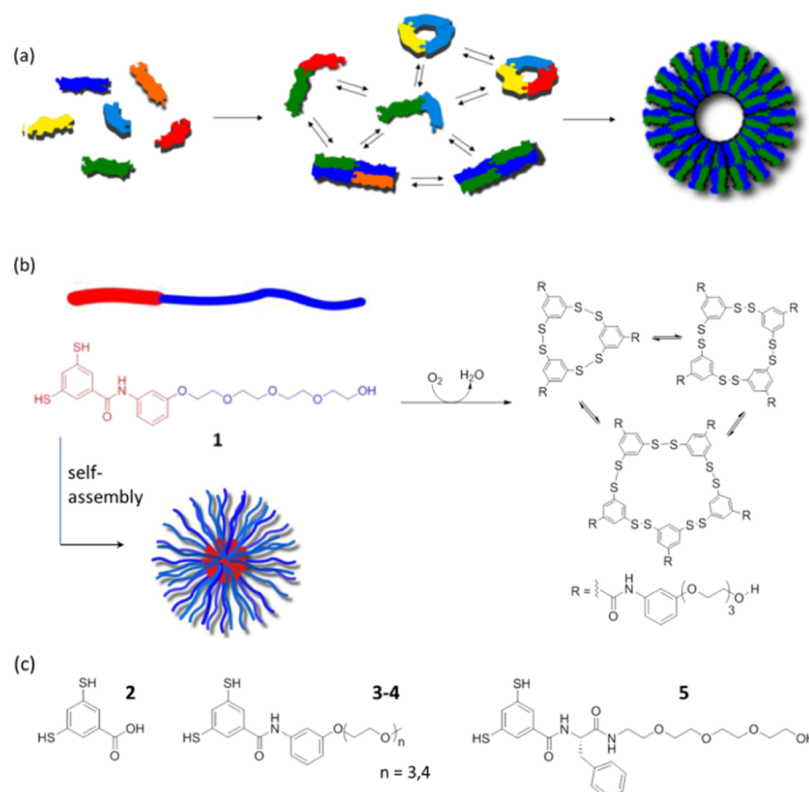


Figure 1. (a) Concept of self-synthesizing materials: self-assembly of one of the oligomers within a DCL shifts the equilibrium in the direction of the very molecule that self-assembles. (b) Amphiphilic dithiol building block **1** self-assembles into micelles or, upon oxidation, gives rise to a set of interconverting disulfide macrocycles. (c) Structures of building blocks **2–5**.

water solubility and renders the overall building block structure amphiphilic.

DCLs were prepared by dissolving building block **1** in borate buffer (50 mM, pH 8.5) in the presence or absence of agitation and allowing the thiols to oxidize by exposing them to oxygen from the air. Agitation was conducted using a magnetic stir bar in order to facilitate a growth-breakage mechanism analogous to that observed previously for self-synthesizing fibers.¹⁵ Analysis by dynamic light scattering (DLS) and transmission electron microscopy (TEM) revealed the presence of micelles in solutions of **1** prior to oxidation of the building block (vide infra). The disulfide macrocycles formed upon oxidation can exchange building blocks through the reaction with residual thiolate anion.³⁵ The composition of the DCLs was analyzed by ultra performance liquid chromatography (UPLC)–mass spectrometry (MS), which uses conditions under which the aggregates fall apart into the molecules from which they are constituted. In contrast to control compound **2**, equipped only with a carboxylate group, which forms a small DCL dominated by cyclic trimers and tetramers (Figure 2a),¹⁵ the DCL made from building block **1** consists of a much larger range of oligomers (Figure 2b; for a nonagitated control, see Figure S1). Thus, a plethora of molecules is available with different amphiphilic properties that can potentially form supra-molecular assemblies. It took several months at room temperature before the final composition of the library was reached (Figure 2c; for the full time-dependent analysis, see Figure S8). This composition did not change further even after several additional months.

While the cyclic tetramer **1₄** is the main product, the library consists of several other species. The broad peak in the UPLC

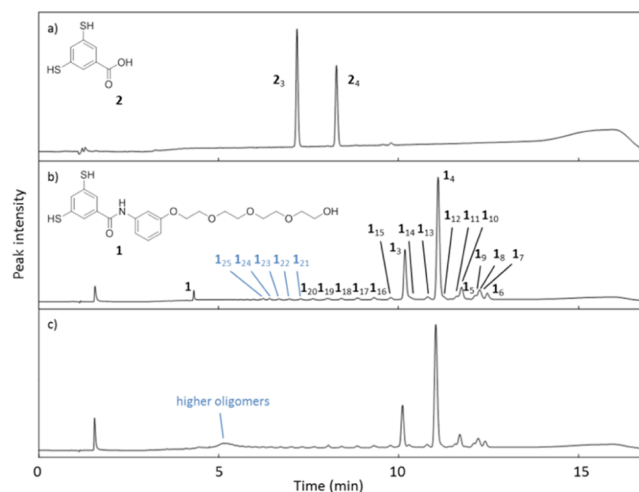


Figure 2. UPLC–MS analysis of a stirred DCL made from 1.0 mM building block **2** or **1** in aqueous borate buffer (50 mM, pH 8.5). Composition of the oxidized libraries made from (a) building block **2** after 7 days; (b) building block **1** after 30 days; and (c) latter DCL after 3 months. Assignments in blue are tentative (low signal-to-noise ratio in the mass spectra precluded direct assignment).

chromatogram eluting at around 5 min most likely contains a collection of high-molecular-weight species.³⁶ The difference in product distribution between the DCLs made from building blocks **2** and **1** suggests that aggregates were formed in the latter. This was confirmed upon analyzing the DCL made from **1** by cryo-TEM (Figure 3). Different membranous structures were observed, among which irregularly shaped vesicles were present (Figure 3a, black arrows), alongside nanosheets (white

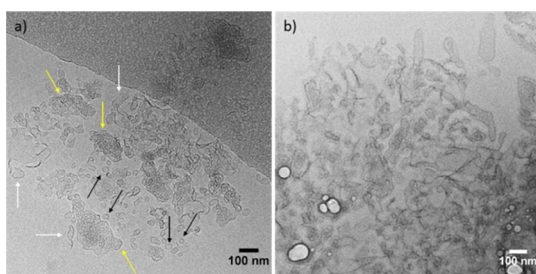


Figure 3. Cryo-TEM micrographs of a library made from building block **1** (1.0 mM) after stirring for 1 month at room temperature (composition shown in Figure 2b). Micrographs (a,b) show membranous structures [vesicles (black arrows), nanosheets (white arrows), and sponge phases (yellow arrows)]. Amorphous aggregates formed in a nonagitated library are shown in Figure S2.

arrows) and sponge phases (yellow arrows). Membranous structures were not observed in nonagitated DCLs, where only amorphous aggregates were formed (Figure S2).

Except for the monomer, all library members were incorporated into relatively large aggregates, as evident from the fact that upon passing a homogenized sample (shaking for 10–20 s by hand to redisperse the material that had settled) through a filter (0.2 μm pore size), only monomer was observed in the filtrate.

Encouraged by the presence of vesicles in the samples, we attempted to further promote their formation. From the fact that also nonvesicular aggregates were formed, we inferred that a mismatch existed between the cross-sectional areas of the hydrophobic and hydrophilic parts of the surfactant molecules. Specifically, the fact that monomer **1** assembles into micelles (vide infra) suggests that the hydrophilic parts of the surfactants occupy a larger cross-sectional area than the hydrophobic parts (packing parameter $p < 1/3$), while for efficient vesicle formation, these cross-sectional areas should be more similar ($0.5 < p < 1$).³⁷

We adopted three strategies to increase the effective packing parameter. First, a number of derivatives (3–5; Figure 1c) of building block **1** were prepared in which either the hydrophilic

or the hydrophobic part of the molecule was changed. In brief, while methylating the ethylenoxide chain (giving rise to **4**) had comparatively little effect on self-assembly, shortening the ethyleneglycol chain by one unit (giving rise to **3**) prohibited the formation of vesicles. DCLs made from building block **5**, which has a more flexible hydrophobic core and an additional amide group, yielded fibrillar structures, rather than vesicles. For further details, see Supporting Information Section 4. We also explored the behavior of DCLs made from mixtures of these building blocks. Similar to the behavior of DCLs made from **1** only, vesicles and sponge phases were observed for mixtures of (i) **1** and **4** and (ii) **1** and **3** and **4**. For further details, see Supporting Information Section 5.

As the alterations in the building block structure did not appear to significantly promote vesicle formation, we explored an alternative strategy that relies on the fact that oligo- and polyethylene glycol-based systems tend to partially desolvate upon increasing temperature, reducing the effective size of the hydrophilic “headgroup”, thereby increasing p .^{38–40} Thus, we monitored the DCLs made from building block **1** at 45 and 65 $^{\circ}\text{C}$ by both UPLC–MS and cryo-TEM. When stirred, these libraries contained similar structures as observed for the DCLs at room temperature. However, the nanosheet-like structures appeared to be less abundant at higher temperatures, and the main aggregates present at 45 $^{\circ}\text{C}$ (Figures 4a–c and S3a–c) and 65 $^{\circ}\text{C}$ (Figures 4d–f and S3d–f) were vesicles and sponge phases. In line with literature observations,⁴¹ the fraction of sponge phases was largest at 65 $^{\circ}\text{C}$. Furthermore, at elevated temperatures, the vesicles had a much more regular shape than those observed at room temperature. Conveniently, the samples oxidized and equilibrated faster at higher temperatures.

Analysis of the composition of the DCLs at different temperatures revealed that the large oligomers are more abundant at higher temperature (Figure S4), suggesting that the proportion of the larger oligomers correlates with sponge phase formation. Interestingly, unlike the nonagitated samples at room temperature, DCLs at higher temperature were also colloidally stable in the absence of stirring. Notably, the shapes

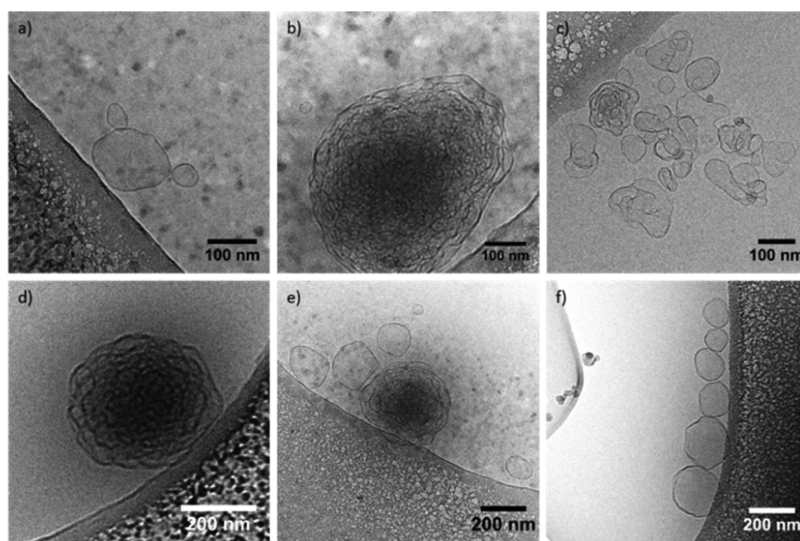


Figure 4. Representative set of cryo-TEM micrographs of a DCL made from building block **1** stirred at 1200 rpm showing different membranous structures: (a–c) 1.0 mM, 45 $^{\circ}\text{C}$ and (d–f) 3.0 mM, 65 $^{\circ}\text{C}$. Additional TEM micrographs are shown in Figure S3. The micrographs show membranous structures, including vesicles (a,c,f) as well as sponge phases (b,d) which appear to coexist (e).

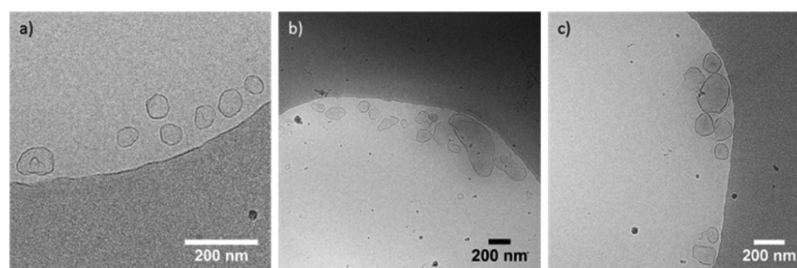


Figure 5. Representative set of cryo-TEM micrographs of a fully oxidized DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 10% of 2-propanol in borate buffer (50 mM, pH 8.5) showing vesicles.

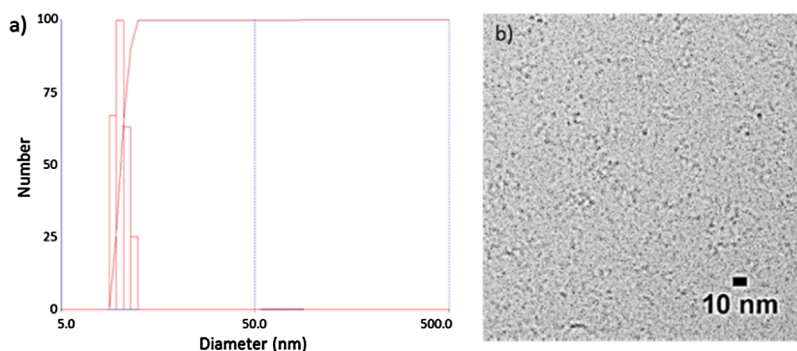


Figure 6. Self-assembly of building block **1** in aqueous solution prior to oxidation. (a) DLS data of a 1.0 mM solution of **1** in aqueous borate buffer (50 mM, pH 8.5) showing the formation of aggregates with a mean diameter of 10 nm. (b) Cryo-TEM micrograph of a solution of building block **1** (1.0 mM) in aqueous borate buffer (50 mM, pH 8.5) showing the formation of micelles (dark spots).

of the aggregates in the nonstirred samples were different from those in the stirred samples, showing folded-up sheets, at both 45 °C (Figure S5) and 65 °C (Figures S6 and S7), in some cases resembling Möbius strips (Figure S7c).

Our final strategy to promote vesicle formation involved the addition of a small fraction of cosolvent. Adding 10% by volume of 2-propanol to a DCL made from **1** induced a slight increase in the fraction of **1**₄ in the library (Figure S8g). Analysis by cryo-TEM showed that vesicles were now the dominant aggregates in these samples, whereas no sponge phases were observed (Figure 5).

The formation of large cyclic oligomers as shown in Figure 2b in DCLs was previously reported for another building block.¹⁷ While in that case the formation of large macrocycles resulted from the aggregation of trimers and tetramers, we ascribe the current behavior to the formation of supramolecular aggregates of monomer **1**, immediately upon dissolution, yielding a high local concentration of thiol groups in the aggregate, facilitating the formation of large disulfide macrocycles. Aggregation of **1** prior to oxidation was evident from DLS (Figure 6a) and cryo-TEM analyses of a freshly prepared solution of **1** in borate buffer. Micelles were observed with an average size of around 10 nm by DLS. Cryo-TEM analysis shows the presence of mostly spherical and a small number of wormlike micelles (Figure 6b).⁴² The fact that building block **1** already assembles into aggregates prior to oxidation facilitates the formation of larger disulfide oligomers (see Figure 2b) as the thiol groups are present in high local concentration.

CONCLUSIONS

In conclusion, DCLs made from building block **1** give rise to a range of membranous structures, including vesicles and sponge phases, whose formation drives the synthesis of the very

molecules that make them. Oligomerization of building block **1**, which initially forms micelles, into macrocyclic disulfides yields library members with a hydrophilic to hydrophobic ratio different from that of building block **1**, which leads to the formation of different types of supramolecular aggregates. The transformation at the molecular level (monomers to oligomers) is thus paralleled by transformations taking place at the supramolecular level (micelles to vesicles and other membranous structures). These results demonstrate the potential of dynamic combinatorial chemistry to access compartments (in the form of vesicles and sponge phases⁴³) that are constituted by a mixture of nontraditional surfactant molecules. Uniquely, this approach enables the direct screening and discovery of assemblies made from mixtures of different surfactants, while integrating the synthesis of these surfactant molecules in the same single step.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b03662.

Materials and methods, synthesis of building blocks **1** and **3–5**; supplementary figures; behavior of DCLs made from **3–5**; behavior of mixed libraries made from **1**, **3**, and **4**; MS spectra; and UPLC–MS characterization of libraries (PDF)

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Notes

The authors declare no competing financial interest.

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(43) Sponge phases are constituted by a complex network of intertwined water-filled channels that, in principle, allow for the diffusion of molecules in and out of their aqueous interior. The potentially slow nature of such diffusion and the extensive surface area of a sponge phase make these an interesting and nontraditional type of compartment, differing from vesicles in the sense that molecules can diffuse in and out without having to cross a membrane.